PAN-PACIFIC ENTOMOLOGIST
Vol. 55, No. 2, pp. 111–116

GENETIC CONFIRMATION OF THE SPECIFIC STATUS OF THE *SPEYERIA ADIASTE* GROUP IN CALIFORNIA (LEPIDOPTERA: NYMPHALIDAE)

STEVEN R. SIMS[1]
Dept. of Entomol., Univ. Calif., Davis, 95616

JOHN G. BRITTNACHER AND FRANCISCO J. AYALA
Dept. of Genetics, Univ. Calif., Davis, 95616

The *Speyeria adiaste* Edwards group is composed of three closely related subspecies occurring in suitable habitats in the Coast Ranges and extreme southern Sierra Nevada of California. These subspecies are well illustrated on Plate 24 in The Butterflies of North America (Howe, 1975). *Speyeria adiaste* was described by W. H. Edwards (1864) and the type locality was fixed by DosPassos and Grey (1947) as the Santa Cruz Mountains, California, where existing populations of *S. adiaste adiaste,* the northernmost and darkest member of the group, occur. *Speyeria adiaste clemencei* (Comstock) has a lighter fulvous ground color and less pronounced dark markings on the upper wing surface than the nominate species (Comstock, 1925). It occurs in the Coast Ranges from Monterey Co. to near the town of San Luis Obispo. The now extinct *S. adiaste atossa* (Edwards) was recorded in the Tehachapi and Tejon Mountains and in the Mt. Pinos region. In overall appearance it resembled its northern counterparts except for a clear yellow-brown ground color and further reduction of the upper surface markings.

These three geographically disjunct subspecies form a color cline ranging from the dark northern *S. adiaste adiaste* to the pale southern *S. adiaste atossa*. Although not yet determined for the *adiaste* group, several other subspecies groups in the genus *Speyeria* (*callippe, coronis, zerene*) display extremely close genetic similarity despite evident phenotypic change (Brittnacher et al., 1978). It is likely that the *adiaste* group fits into this pattern which suggests a rather recent evolutionary divergence of the different color forms.

The distributional limits of these subspecies seem, in part, to be determined by the availability of their violet (*Viola*) food plants and by desiccation tolerances of first instar larvae to dry diapause period (summer–fall) conditions (Sims, unpublished data). Xeric conditions tend to limit distributions of many violet species and prove fatal to species lacking adequate desiccation resistance. *Speyeria adiaste* may once have occurred in an unbroken range throughout the coastal mountains. Division of the range was

possibly influenced by the drier climatic conditions of the Pliocene (Axelrod, 1948) or the pluvial periods of the Pleistocene. The warmer and drier post-Pleistocene conditions would have supplemented the process of range limitation.

Since the original description, the taxonomic status of *S. adiaste adiaste* and the later named subspecies has been in doubt. The most recent systematic treatment of the genus *Speyeria* (DosPassos and Grey, 1947) regards the *adiaste* complex as a subspecies of *S. egleis* (Behr). Although *S. egleis* exhibits a much wider distribution than *S. adiaste,* the groups are (or were) completely allopatric except in the Tehachapi Mountains. In this latter location, the species populations were spatially isolated by elevation, *S. egleis* preferring the higher peaks and slopes while *S. adiaste atossa* frequented mid-elevation habitats. Other interspecific barriers to reproduction might well have included differences in species specific pheromones or mating behavior (Magnus, 1958).

Phenotypically, specimens within populations of the three taxa in the *adiaste* group are quite uniform in contrast to many other species in the genus (Moeck, 1957). *Speyeria egleis* often exhibits remarkable intrapopulation variation both in coloration of the disc (basal undersurface of hind wing) and in the silvering or absence of silvering of the hind wing spots.

The taxonomic relationship of *S. adiaste* has recently undergone another shuffling in which the group regained specific status (Emmel and Emmel, 1973; Howe, 1975). The purpose of this paper is to present genetic evidence which ex post facto justifies this latest separation of the *S. adiaste* group.

Genetic Differentiation

Gel electrophoretic techniques are well known and currently widely used to study inter- and intraspecific levels of genetic variability in diverse groups of organisms. Techniques of gel electrophoresis and enzyme assay allow identification of allelic variation at single gene loci. When data are obtained from a "moderate" number of enzyme loci, the results, with certain assumptions, may be extrapolated to the genome as a whole. The loci studied are assumed to represent a random sample of the genome with respect to allelic variation. Possible sources of bias in such an assumption have been cited by Lewontin and Hubby (1966) and Ayala et al. (1970). It has been amply demonstrated that gel electrophoresis is an extremely valuable systematic tool (see Avise (1974) for review). Ayala (1973) and Ayala and Dobzhansky (1974) have used allozyme data as diagnostic characters for subspecies of *Drosophila willistoni* and *D. pseudoobscura.*

We examined genetic variation at 16 loci in all ten species of *Speyeria* occurring in California plus several of the subspecies existing in the *callippe, coronis, hydaspe,* and *zerene* groups (Brittnacher et al., 1978). With few

Table 1. Genetic similarity (above diagonal) and genetic distance (below diagonal) for seven species in the genus *Speyeria*.

	1	2	3	4	5	6	7
1. *adiaste*		.775	.866	.798	.852	.917	.762
2. *atlantis*	.255		.913	.954	.950	.801	.922
3. *callippe*	.144	.091		.933	.985	.881	.901
4. *coronis*	.225	.047	.069		.938	.883	.903
5. *egleis*	.161	.051	.015	.064		.872	.938
6. *hydaspe*	.087	.222	.126	.125	.138		.790
7. *zerene*	.272	.081	.104	.102	.064	.236	

exceptions, only males were used in the assays. Females were used in a companion study of the reproductive biology of the genus.

Populations of *S. egleis egleis* from the following Sierra Nevada locations were analyzed: Bowman Lake, Nevada Co., el. 1700 m (n = 8); Donner Pass, Placer Co., el. 2100 m (n = 5); and Yuba Pass, Sierra Co., el. 2000 m (n = 10). Two populations of *S. adiaste clemencei* were sampled: Arroyo Seco Camp, el. 260 m (n = 19) and Chew's Ridge, el. 1100–1500 m (n = 45), both in the coast range of Monterey Co. California.

Speyeria adiaste clemencei and *S. egleis egleis* were found to have two fixed differences at the sixteen loci studied. These were for glyceraldehyde-3-phosphate dehydrogenase and glucose-6-phosphate dehydrogenase (see Brittnacher et al., 1978, for details).

The amount of genetic differentiation between *S. adiaste* and *S. egleis* is substantial when compared to the genetic differentiation between other species of *Speyeria*. Table 1 summarizes the differentiation found in *Speyeria* using Nei's (1972) method of calculating genetic distance, *D*, and genetic identity, *I*. It can be seen that the genetic distance between *S. egleis* and *S. adiaste* is greater than the distance between *S. egleis* and *S. atlantis*, *S. callippe*, *S. coronis*, and *S. zerene*. It is thus unlikely, based on this genetic evidence alone, that the members of the *S. adiaste* group are subspecies of *S. egleis*.

Karyotype Determination

Chromosome numbers of *S. adiaste clemencei* and *S. egleis egleis* were determined in view of the potential for additional substantiation of the allelic differences. Chromosome counts were made in 19 nuclei in testes of 6 *S. adiaste clemencei* males field collected at Chew's Ridge on June 11, 1974. Counts were similarly made in 10 nuclei in testes of 3 *S. egleis egleis* pupae derived from the population at Loon Lake, El Dorado Co., California.

Cytological techniques followed for *S. adiaste clemencei* involved fixa-

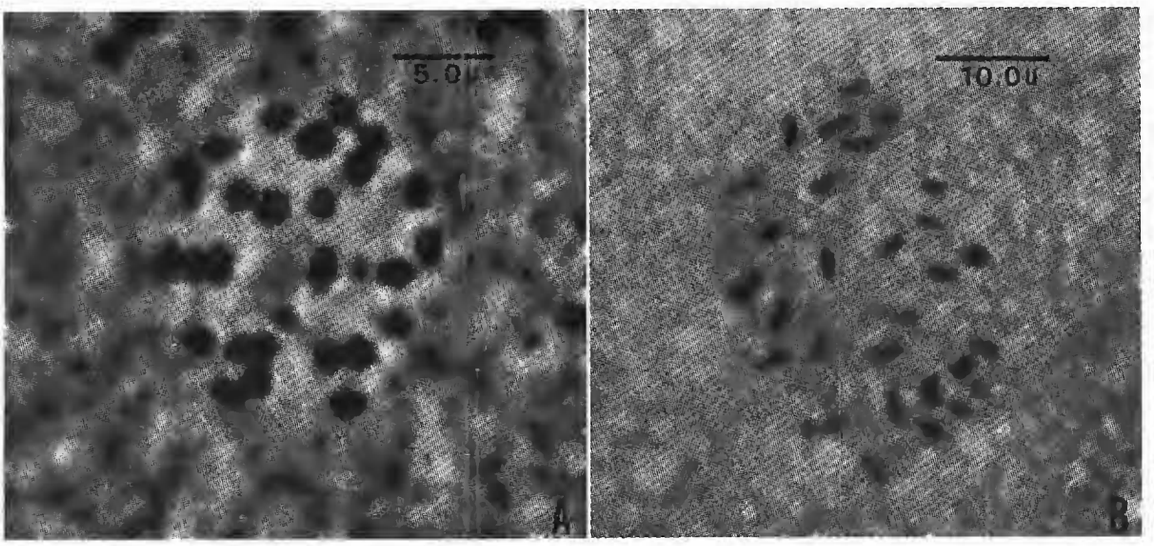

Fig. 1. Chromosomes of *Speyeria*, (A) *S. adiaste clemencei*, N = 29, metaphase; (B) *S. egleis egleis*, N = 29, metaphase.

tion for 5 minutes in a 3:1 absolute ethanol:glacial acetic acid solution, staining with 0.5% lacto-acetoorcein, and squashing on a slide using hand pressure (Emmel, 1968). A modified squash-air dry technique described by Goodpasture (1976) was used for chromosome counts of *S. egleis egleis*. Preparations were examined under oil using phase contrast illumination at a magnification of 960×. Photographs were taken on Kodak High Contrast Copy 35 mm film at a film plane magnification of 400×.

In all sufficiently clear preparations, the haploid number (N) was found to be 29 for both *S. adiaste* and *S. egleis* (Fig. 1). This count is identical to the majority of previously determined species in the genus (Maeki and Remington, 1960). Exceptions occur in the *S. callippe* and *S. coronis* groups where apparently N = 30. Curiously, *S. callippe* and *S. egleis* are cytologically distinct despite having the highest genetic similarity value (.985) of all species studied.

Immature Stages

Little other comparative biological data is available on the *S. adiaste* and *S. egleis* groups. Edwards (1897) described and illustrated the life history of *S. egleis* from Colorado. Comstock and Dammers (1931) described the mature larva and pupa of *S. adiaste atossa* from Lebec, Kern Co., California. Adequate distinguishing characters cannot be determined from the descriptions except for the slightly larger mature size of the *S. adiaste atossa* larva (35 mm vs. approx. 31 mm for *S. egleis*) which may simply be a sex-related difference. We believe it significant to note that mature larvae of both are characterized by an irregular yellowish patch on the dorsum of the head capsule, a trait missing in California *S. callippe* and *S. coronis*.

Summary

Speyeria adiaste clemencei, long considered a member of the *S. egleis* group, shows a relatively low degree of genetic similarity (I = .852) to this species. Two fixed differences were present among sixteen loci studied. From a perspective of genetic relationships in other *Speyeria* this divergence appears significant at the species level. The marked phenotypic distinctiveness of the *Speyeria adiaste* group, uniformity of phenotype within populations, geographic isolation, and lack of hybridization are additional factors arguing for recognition of specific distinction. The chromosome number (N = 29) of both *S. adiaste* and *S. egleis* is similar to the majority of other *Speyeria* and does not provide an adequate index of relationship. Both groups have similar immature stages with only minor color differences. In these two species, it appears that allozyme characters are better differentiated than chromosome number or immature stage morphology.

Acknowledgments

We thank Sterling O. Mattoon for providing pupae of *S. egleis egleis* and Dr. A. M. Shapiro for comments on the manuscript.

Literature Cited

Avise, J. C. 1974. Systematic value of electrophoretic data. Syst. Zool. 23: 465–481.

Axelrod, D. I. 1948. Climate and evolution in western North America during middle Pliocene time. Evolution 2: 127–144.

Ayala, F. J. 1973. Two new subspecies of the *Drosophila willistoni* group (Diptera: Drosophilidae). Pan-Pac. Entomol. 49: 273–279.

Ayala, F. J., and T. Dobzhansky. 1974. A new subspecies of *Drosophila pseudoobscura* (Diptera: Drosophilidae). Pan-Pac. Entomol. 50: 211–219.

Ayala, F. J., C. A. Mourão, S. Pérez-Salias, R. Richmond, and T. Dobzhansky. 1970. Enzyme variability in the *Drosophila willistoni* group. I. Genetic differentiation among sibling species. Proc. Nat. Acad. Sci., USA 67: 225–232.

Brittnacher, J. G., S. R. Sims, and F. J. Ayala. 1978. Genetic differentiation between species of the genus *Speyeria* (Lepidoptera: Nymphalidae). Evolution 32: 199–210.

Comstock, J. A. 1925. Studies in Pacific Coast Lepidoptera. Bull. So. Calif. Acad. Sci. 24: 3–4.

Comstock, J. A., and C. M. Dammers. 1931. Notes on the early stages of four California Argynnids (Lepid.). Bull. So. Calif. Acad. Sci. 30: 40–48.

DosPassos, C. F., and L. P. Grey. 1947. Systematic catalogue of *Speyeria* (Lepidoptera, Nymphalidae) with designations of types and fixations of type localities. Amer. Mus. Nov. 1370. 30 p.

Edwards, W. H. 1864. Notes on the Argynnides of California. Proc. Entomol. Soc. Phil. 3: 434–436.

Edwards, W. H. 1897. Butterflies of North America. Houghton Mifflin and Co., New York. Ser. 3, pt. IX.

Emmel, T. C. 1968. Methods for studying the chromosomes of Lepidoptera. J. Res. Lepid. 7: 23–28.

Emmel, T. C., and J. F. Emmel. 1973. The butterflies of Southern California. Nat. Hist. Mus. of Los Angeles Co., Sci. Series 26. 148 p.

Goodpasture, C. 1976. High-resolution chromosome analysis in Lepidoptera. Ann. Entomol. Soc. Amer. 69: 764–771.

Howe, W. H. 1975. The butterflies of North America. Doubleday & Co., New York. 633 p.

Lewontin, R. C., and J. L. Hubby. 1966. A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. Genetics 54: 595–609.

Maeki, K., and C. L. Remington. 1960. Chromosomes of North American Lepidoptera. Part 4. J. Lepid. Soc. 14: 179–201.

Magnus, D. 1958. Experimental analysis of some "overoptimal" sign-stimuli in the mating behaviour of the fritillary butterfly *Argynnis paphia* L. (Lepidoptera: Nymphalidae). Proc. 10th Int. Congr. Entomol. 2: 405–418.

Moeck, A. H. 1957. Geographic variability in *Speyeria*. Comments, records and description of a new subspecies. Privately printed, sponsored by Milwaukee Entomol. Soc. 48 p.

Nei, M. 1972. Genetic distance between populations. Amer. Natur. 106: 283–291.

Footnote

[1] Current Address: Dept. of Biol., Univ. Notre Dame, Notre Dame, IN, 46556.